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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 6 /00

A2

(11) International Publication Number: **WO 00/03747**

(43) International Publication Date: 27 January 2000 (27.01.00)

(21) International Application Number: PCT/IT99/00224

(22) International Filing Date: 16 July 1999 (16.07.99)

(30) Priority Data:
RM98A000476 17 July 1998 (17.07.98) IT

(71)(72) Applicants and Inventors: DOLCI, Giovanni [IT/IT]; Via Antonio Guarnieri, 51, I-00124 Roma (IT). MONGIORGI, Romano [IT/IT]; Via Canovella, 3/2, I-40043 Marzabotto (IT). PRATI, Carlo [IT/IT]; Via Mameli, 18, I-47100 Forli (IT). VALDRE', Giovanni [IT/IT]; Via Mascarella, 77/2, I-40126 Bologna (IT).

(74) Agents: BANCHETTI, Marina et al.; Ing. Barzanò & Zanardo Roma S.p.A., Via Piemonte, 26, I-00187 Roma (IT).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

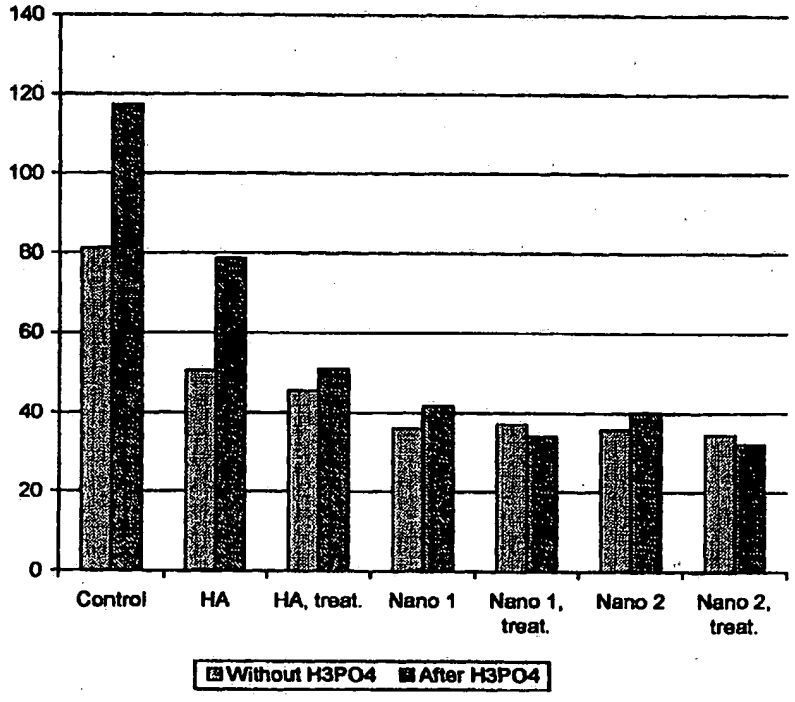
Published

Without international search report and to be republished upon receipt of that report.

(54) Title: ODONTOSTOMATOLOGIC USE OF APATITE-BASED NANOSTRUCTURED MATERIALS

(57) Abstract

Biocompatible nanocrystalline materials based on apatite with high specific surface properties, having average size of the crystallites comprised between 0.5 and 200 nm and possible lattice deformations and/or defects, are proposed for use in the fields of dentistry and dental hygiene, in particular for the production of preparations, solutions, toothpastes and materials for the remineralisation of enamel and dentine and for the treatment of dentine hypersensitivity. The apatite-based nanostructured materials appear to possess specific physico-chemical properties connected to their nanocrystalline and defective nature, such as the remarkable surface chemical reactivity and the hygroscopicity, which allow the material to recrystallise as a result of the metastable character of the nanocrystalline system, to be used for the remineralisation of enamel and dentine. In addition, they possess the most suitable grain sizes to easily and deeply penetrate mechanically within the dentinal tubules, and also cause it to cement the internal surface thereof, both by swelling and by readily reacting with the natural tissues.



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ODONTOSTOMATOLOGIC USE OF APATITE-BASED NANOSTRUCTURED MATERIALS

SPECIFICATION

The present invention concerns the use in odontostomatology of apatite-based nanostructured materials. More specifically, the invention concerns the application in the fields of dentistry and dental hygiene of biocompatible crystalline products based on apatite with high specific surface properties, having single constituent crystals of nanometric size. Such products are particularly useful in the treatment of dentine hypersensitivity and in the remineralisation of dental tissue.

As it is known, dentine sensitivity or hypersensitivity is a complex symptomatology, characterised by pain and sensitivity to thermal, mechanical (i.e. tactile), chemical and osmotic stimuli caused by exposure of the dentin to the oral environment. Several data on the epidemiology of dentine hypersensitivity have been published in the latest years, revealing, *inter alia*, that such clinical condition affects more than 15% of the population in the age range between 20 and 40 years, with a particular preference for females.

Dentine hypersensitivity may be primary, i.e. not resulting from any therapeutic intervention on the dental components, or it may be secondary to previous periodontal interventions and/or to injuries resulting in dentine exposure, or to restorations (including dental prostheses) with projecting margins or having an incorrect shape. After an extensive periodontal intervention, especially of resective surgery, patients quite frequently report a hypersensitivity not previously present. In such cases the affection is also referred to as radicular or cervical hypersensitivity.

According to the theory advanced by Brännström (i.e., the hydrodynamic theory of pain induction; see M. Brännström, A. Astrom: The hydrodynamics of the dentin: its possible relationship to dentinal pain. Int. Dent. J., 22:219-227 (1972)) and, later, by Pashley (D.H. Pashley, Mechanisms of dentin sensitivity. Dent. Clin. North Am.; 34: 449-473 (1990)), the presence of

exposed dentine and of open dentinal tubules allows the dentine fluid to move within the said tubules. Such displacement is induced by mechanical stimuli (such as, for instance, by passing the specillum over the tooth surface), by osmotic and chemical stimuli (such as by the contact with food, beverages, etc.) and by thermal stimuli (both hot and, specially, cold). However, it has not been ascertained, so far, whether the displacement of the dentine fluid causes a nervous stimulation (for instance, a stimulation of intratubular nerve fibres) or whether it directly stimulates the odontoblasts projections (which occupy about 2/3 of the whole tubule length).

In all cases, the greater is the exposed dentine surface (with non-obstructed tubules), the greater is the likelihood that the patient be sensitive to any stimuli. In other terms, the exposed dentine behaves as a semi-permeable membrane, wherein the dentine tubules represent the membrane pores. Therefore, the diameter and number of the exposed tubules largely affect the degree of sensitivity, as it has been ascertained *in vivo* by Yoshiyama et al. (M. Yoshiyama, Y. Noiri, K. Oaki, A. Uchida, Y. Ishikawa, H. Ishida, Transmission electron microscopic characterisation of hypersensitive human radicular dentin, J. Dent. Res.; 69: 1293-1297 (1990)).

As a direct clinical consequence of the foregoing, any dental treatment involving the removal of enamel and dentine, and thus the opening of the dental tubules, unavoidably results in an increased sensitivity. Accordingly, the patient with dentine hypersensitivity is found to have, at the same time, an area of exposed dentine (i.e., an area deprived of enamel and/or of gum covering) with open tubular orifices, and no kind of surface debris or smear layer (the latter term actually meaning the layer of debris that normally covers the dentine and closes the tubules with plugs, i.e. the so-called smear plugs) obstructing, at least partially, the dentine tubules.

Food may play a primary role in causing the onset or, more properly, the becoming acute again, of dentine hypersensitivity. As a matter of fact, several food items or beverages have acid properties, as they contain citric, phosphoric or maleic acid, and they may easily effect the chemical removal of the surface smear layer, thus opening the dentine tubules. Also other food

items or beverages, such as red wine, citrus fruits and unripe fruits in general, have been reported to have effects on the dentine hypersensitivity. In addition, as pointed out before, a particular situation may occur in patients who have undergone a resective periodontal intervention, about 80% of whom reports dentine sensitivity after the periodontal therapy. Also other periodontal procedures may be related to the occurrence of dentine hypersensitivity: for instance, during the operative procedures of dental calculus removal, root scalling, root planning, root polishing and surface cleaning, any residual cement (which is often already absent) and any infected dentine are removed, thus resulting in the removal of large root surfaces and in the exposure of an extremely high number of open dentine tubules. In the event that such exposure allows the passage of intratubular fluid from the interior of the pulp cavity to the exterior thereof, with loss of plasma proteins and electrolytes, and, viceversa, the passage of liquid and toxins from the exterior of the pulp cavity to the interior thereof, a temporary pulpitis occurs, accompanied by a painful symptomatology. The latter is normally felt after some hours or days from the intervention. Very often, the symptoms spontaneously decrease owing to the spontaneous obstruction of the tubules, resulting from the deposition of bacterial debris, the precipitation of proteins and fibrinogen and the production of a smear layer by the patient himself during the normal dental hygiene procedures with the toothbrush (Pashley, 1990, *loc. cit.*).

Since the presence of open dentine tubules represents the critical stage of dentine hypersensitivity, the most reliable therapy aims, basically, at occluding the said tubules. A different therapeutic approach, aiming at reducing the receptor sensitivity, consists in increasing the intratubular concentration of potassium and strontium ions (by means of the application of dentifrice or other agents), in order to make the dentinal nerve fibres less excitable. However, the hypothesis of an activity of the K^+ and Sr^{2+} ions in influencing the nerve stimulus conduction has found limited clinical confirmation (D.G. Gillam, J.S. Bulman, R.J. Jackson, H.H. Newman, Efficacy of a potassium nitrate mouthwash in alleviating cervical dentine sensitivity (CDS), J. Clin. Periodontol. 23: 993-997 (1996)).

As far as home-therapy is concerned, several dentifrices and gels specific for sensitive teeth are commercially available, containing, for instance, strontium chloride hexahydrate, potassium nitrate or citrate or stannous fluoride, and also some mouthwashes and periodontal gels have been proposed. Data reported in the literature show that several toothpaste products possess some desensitising action, which is often independent of the kind of active ingredient employed (D.G. Gillam, J.S. Bulman, R.J. Jackson, H.N. Newman, Comparison of 2 desensitizing dentifrices with a commercially available fluoride dentifrice in alleviating cervical dentine sensitivity, J. Periodontol., 67: 737-742, (1996)). Very often, also the other components of the toothpaste play an important role, in that they are able themselves to seal the dentine tubules. In addition, the placebo effect plays a role considered by someone to be a primary one.

The most studied and well-known products for home therapy include dentifrices containing strontium chloride hexahydrate. The latter, as pointed out in the foregoing, is used in view of its ability of closing the dentine tubules, rather than for its activity in depressing the dentinal nerve fibres stimulation. It is believed that the Sr^{2+} ion reacts with the apatite matrix of the dentine, thus forming with said matrix an insoluble strontium-apatite. The latter settles as a layer of microcrystals, that are able to reduce the functional diameter of the dentine tubules. Also stannous fluoride is believed to act by forming insoluble complexes with dentine (i.e. fluoroapatite or calcium fluoride), which complexes are able to obstruct the tubular lumen. As far as potassium citrate is concerned, it has been shown that the citrate ion chelates the calcium contained in the tooth hydroxyapatite, thus resulting in the formation of a soluble calcium citrate (Misra D.N., Interaction of citrate Acid with Hydroxyapatite: Surface exchange of ions and precipitate of calcium citrate, J. Dent. Res., 75(6), 1418-1425, June 1996).

In this connection, other dentifrices and desensitising preparations have been proposed, which, rather than being based on products that form insoluble precipitates by reacting in the oral environment, are based on the direct use of apatite, i.e., the basic material making up the dentine, in the

microcrystalline state. For instance, the US patent No. 4634589 (Wurttembergische Parfumerie-Fabrik) discloses the use of a crystalline apatite having a particle size of less than 8 μm as an abrasive and polishing agent, active in the treatment of dentine hypersensitivity and also having a remineralising action on the dental tissue. The dentifrice formulation proposed contains at least 15% by weight of such ingredient. Similarly, the European patent application No. 0346957 (Unilever) discloses the use of hydroxyapatite as a desensitising abrasive agent for dental products. In this case, the hydroxyapatite particle size is 1-15 μm, and the formulation proposed also comprises a source of potassium and/or strontium (as a further desensitising agent).

The treatment of dentine hypersensitivity by home therapy, however, has the drawback of requiring long periods of time (often more than four weeks) to reach any appreciable clinical results. In addition, the said results are often only partial, as a great amount of co-operation is required from the patient. In view of that, the treatment of dentine hypersensitivity is preferably carried out at the dentist's consulting room, by directly treating the affected tooth (or teeth). Also in this case, the main aim is to occlude the open dentine tubules. Several techniques and materials have been proposed to that aim, according to the degree of seriousness of the symptoms. Such techniques can be schematically divided into four main groups: 1) mechanical treatment of the sensitive surface in order to obtain a new smear layer; 2) application of agents effective in occluding the dentinal tubules, either directly or by forming insoluble precipitates; 3) impregnation and obstruction of the dentine tubules by means of adhesives; 4) application of dental restoration materials, optionally together with intraoral devices for sustained release of fluorine. In the most serious cases, as well as in those cases where any other kind of therapy is ineffective, the dental restoration and the endodontal treatment are often included in order to finally solve the problem.

As regards the first one of the treatments mentioned above, since dentine is not permeable when a suitable smear layer is present, it is often possible, by simply applying an abrasive paste (such as the Nupro paste) and by working the dentine surface by means of silicone or rubber cups, to obtain

a thin and uniform smear layer and, consequently, the obstruction of the tubules. Although simple, such treatment does not give long-lasting results, as the smear layer is soluble (specially in an acid environment), and therefore it may be easily removed by the patient. An incorrect brushing of the teeth or the ingestion of acid liquids (such as fruit juices, beverages, etc.) are often sufficient to cause the smear layer removal. Therefore, the adoption of the above treatment is limited to non serious cases, e.g. to treat patients after a periodontal intervention with exposure of limited dentine surfaces and without any previous history of dentine hypersensitivity.

The second kind of medical treatment mentioned in the foregoing consists, for instance, in applying oxalates (such as potassium, iron or aluminium oxalate, and oxalic acid) on the affected dentine surface. Such agents may react with the dentinal apatite constituents, thus forming insoluble calcium oxalate microcrystals on the surface and in the interior of the dentine tubules. The solutions containing the concerned agent may easily be applied on the dentine surface by means of little brushes, and they may be left *in situ* for 2-3 minutes, this period of time being enough for the said microcrystals to form. It is to be noted, however, that this technique may involve some problems due to the toxicity of oxalates. Also silver nitrate, another agent employed for a long time for the same purpose, has a mechanism of action based on the formation of precipitates (Ag chloride) which occlude the dentine tubules.

The same kind of therapeutic approach also includes the direct application of microcrystalline apatite, as mentioned in the foregoing in connection with products for home therapy. More specifically, the medical treatment includes the use of an ultramicronised apatite, such as that disclosed, for instance, in the Italian patent No. 1271874, where the particle size of about 70% of the apatite microcrystals is less than 1 μm (0.2-1 μm). Such product is considered to be more active than the micronised apatite products disclosed in the foregoing, on the basis of the simple consideration that the dentine tubules have an average diameter of about 1.3-3.5 μm. Therefore, crystals having slightly submicronic size may, theoretically, penetrate within the dentine tu-

bules, thus performing a better mechanical occlusion. On the contrary, the micron-sized crystals disclosed in the two previously cited patent documents may only create an occlusive layer on the dentine surface, without penetrating into the tubules.

Considering the medical treatments, however, the procedures belonging to the third group referred to above, consisting in dentine impregnation by means of adhesive resins, are considered to be more effective. The resin impregnation technique, proposed by Nordenvall and Brännström (K.J. Nordenvall, M. Brännström, In vivo resin impregnation of dentinal tubules, J. Prosthet. Dent.; 44:630-637 (1980)), has become extremely reliable only after the introduction of dentinal adhesive systems consisting of highly hydrophilic resins, which are able to infiltrate through the dentine and to penetrate within the tubules for some tens of microns. It has been shown that the dentine surface, treated with etching acid (for instance, with phosphoric or malic acid) becomes demineralised up to 4-10 µm of depth, and rich in partially collapsed collagen fibres only. This treatment enhances the penetration of the resin (usually, a primer) within the dentine tubules and within the collapsed collagen. The resin, by penetrating into the tubules, forms long plugs (referred to as resin tags) which occlude the tubular lumen and reduce the dentine permeability. In addition, the resin impregnates and occludes the small lateral channels as well (as it has recently been confirmed by some studies by scanning electron microscopy), thus contributing to the formation of a thick resin network effective in stably blocking any movement of the dentine fluid. A *hybrid layer* made of resin and collagen is thus formed. From the point of view of the mechanism of action, it is not clear whether other factors co-operate in reducing the sensitivity, in addition to the tubules occlusion and the consequent permeability reduction (which is, very likely, the main mechanism of action). It has been hypothesised that some of the components of the dentinal primers also have a pharmacological action such as to reduce the symptoms.

At the present state of knowledge, the impregnation treatment of the exposed dentinal surface with resin is to be considered specific for patients wherein few dental elements show a medium or serious dentine hypersensi-

tivity.

Finally, in the occurrence of widespread and serious dentine hypersensitivity, a distinction has to be made between lesions with deficiency of periodontal tissue, wherein surgery represents the treatment of choice, and lesions with extensive enamel loss, wherein a conservative dental restoration is indicated. For the latter purpose, the same dentinal adhesive systems of the previously disclosed method may be used, followed, obviously, by the application of a composite resin-restorative material; in the alternative, photocuring glass-ionomer cements may be used. This kind of intervention, as pointed out before, is reserved to cases showing a marked deficiency of tissues, or when the hypersensitivity is extremely serious. The further therapeutic step is, obviously, the endodontic treatment of the affected tooth.

In addition to the well established treatments referred to in the foregoing, further literature, mainly patent publications, proposes (besides the micronised and ultramicronised apatite materials mentioned above) the use of amorphous calcium phosphates, both in order to remineralise (and, optionally, to fluoridate) the dental tissue, and in order to reduce the dentine hypersensitivity. The US patent No. 5268167 (American Dental Association Health Foundation) proposes, to such aim, the use of amorphous calcium phosphates (i.e., ACP or amorphous calcium phosphate; ACPF or amorphous calcium fluorophosphate; ACCP or amorphous calcium carbonate phosphate). According to the disclosure, the said amorphous salts, or solutions that may originate such salts by precipitation, are applied on the dental tissue surface, and settle thereon and within the same tissue. The amorphous salts are then converted to crystalline apatite, thus performing a remineralising action and reducing dentine sensitivity. In the same frame, the PCT patent application No. WO 94/04460 (American Dental Association Health Foundation) also presents the ACCPF phosphate (i.e., amorphous calcium carbonate fluorophosphate), as a new compound particularly interesting for the same purpose.

In the light of the prior art referred to above, it is an object of the present invention to provide a biocompatible product substantially based on apatite, able to satisfactorily comply with various therapeutic requirements in the

odontostomatologic field and, firstly, to effectively and lastingly counteract dentine hypersensitivity, by deeply occluding and cementing the dentine tubules, and by exerting a desensitising action that withstands the attack of any chemically aggressive agent present in the oral environment, such as acid beverages and food.

In accordance with the invention, it has been found that the apatite materials characterised by a nanocrystalline and/or defective structure (as opposed to the known amorphous and microcrystalline structures, including the ultramicronised forms) lend themselves much better to the objects mentioned above, as they allow to achieve remarkable therapeutic results, up to the complete suppression of dentine hypersensitivity and, in addition, with a considerable remineralisation of the dental tissue. Such results are made possible by the unique features of the crystalline nanostructures, that substantiate the great potential of these advanced materials in many applicative fields.

In general, the nanocrystalline materials are artificially synthesised materials, characterised by a constituent phase, or by granular structures, modulated on a length scale normally smaller than 100 nm. According to the number of dimensions in which they show a nanometric structure, the nanocrystalline materials are considered to be with dimensionality equal to zero (clusters of atoms – for instance, dispersed in a non-nanocrystalline matrix , filaments or tubules), with dimensionality one (multilayers, i.e., layers which are nanometric in the only direction of the thickness), with dimensionality two (granular superpositions, ultrafine, or buried layers), or with dimensionality three (nanophasic materials, wherein all of the constituent phases are of nanometric proportions on three dimensions) (R.W. Siegel, in Materials Science and Technology, Vol. 15: Processing of Metals and Alloys, R.W. Cahn, 583 (1991)). The particular properties of the nanocrystalline materials in comparison with the conventional materials result from the combination of their three basic and distinctive features, namely: i) atomic domains (i.e., clusters, grains, layers or phases) limited in space to less than 100 nm; ii) significant atomic fractions associated with interface environments (that is, grain bounda-

ries, interfaces and heterophase, and free surfaces); and iii) interactions among their constituent domains.

At present, it is possible to produce inexpensively, by means of several physical and chemical processes, clusters of atoms in the range of the nanometric dimensions, containing from hundreds to tens of thousands of atoms, in such a number as to be assemblable into materials which advantageously incorporate into one single material a multiplicity of effects due to the dimension. The said effects range from the electronic effects of quantum dimension caused by the spatial confinement of the valence electrons, to the suppression of mechanisms of lattice defects, such as the dislocation generation and the migration towards limited granular dimensions. The basis of the particular performances of the nanostructured materials is to be found in the fact that a physical property of matter becomes altered when the entity or the mechanism responsible for such property (or the combination thereof) are confined within a space (defined by the dimension of the atoms set) smaller than a given critical length associated with such entity or mechanism. Therefore, for instance, a metal that is conventionally ductile owing to the usual ease in creating and displacing dislocations through the crystal lattice thereof will become remarkably harder when the grain size is reduced down to a critical point wherein the dislocation sources are no more able to work at the low levels of the applied stress.

In addition to being characterised by the dimensions of their ultrafine domain (i.e. grains or layers), the nanocrystalline materials are also characterised by the high number of interfaces they contain. Since the number of interfaces present in the nanocrystalline materials is much higher than in the conventional materials, a suitable control, in the course of the synthesis, on the nature of the interfaces created between the constituent phases leads to a control on the nature of the interactions through the said interfaces. In order to have an idea of the importance of the interface environment in a nanocrystalline material it is sufficient to consider, for instance, that in a material with an average grain size of 5-10 nm the percentage of atoms comprised in the grain boundaries is in the range from 15 to 50%.

In view of the general features of the nanocrystalline materials referred to in the foregoing, for the use proposed according to the invention the apatite-based nanostructured materials appear to possess, firstly, the most suitable granular dimensions to easily and deeply penetrate within the dentine tubules (which, as pointed out before, have diameters in the range of 1.3-3.5 μm). In addition, as pointed out before, the concerned materials show specific physico-chemical properties connected with their nanocrystalline nature, such as the remarkable surface chemical reactivity and the hygroscopicity, which not only allow the material to penetrate mechanically within the tubules, but also cause it to cement the internal surfaces thereof, both by swelling and by readily reacting with the natural tissue, and recrystallising as a result of the metastable character of the nanocrystalline system. Actually, the latter may have, in this specific case, a high defective content.

Apatite-based materials characterised by a nanocrystalline structure appear to have somehow been described, so far, only in the PCT patent application No. WO 97/17285 (Etex Corp.), concerning the low temperature synthesis of a low crystallinity apatite for use in bone tissue graft. Actually, the definition of "low crystallinity material" given in such text generically includes both amorphous materials and nanocrystalline materials (with nanometre-sized or Ångstrom-sized crystalline domains). Such document, however, exclusively concerns the production of resorbable synthetic bone materials, which, in view of their intended use, are formed in moulds to give solid elements. The only relevant requirement for such use is the ability of closely reproducing the natural bone tissues, so that the synthetic bone graft is integrated and resorbed in the said tissues. The applications of the concerned material in the orthopaedic field differ from the odontostomatological application in that in the first case the material must comply with stability requirements, it must be able to easily integrate within the bone and, in some cases, it must be able to induce bone growth (direct relationship between material and cells), while in the second case the material must interact with mineral tissue having chemical and structural properties different from bone, and having a different organic component. Consequently, in the latter case character-

istics of the material such as surface reactivity and metastability will have to prevail. Furthermore, the orthopaedic applications that involve interventions on the bone tissue are concerned with a system interacting with the blood stream and unaffected by variations induced by the contact with the external environment (such as pH changes or compositional variations of various kinds). This is right the opposite of what happens in the oral environment, and on the tooth surface.

Accordingly, the present invention specifically provides the use of apatite-based nanostructured materials of the general formula:

$$Ca_{10-x}M_x(PO_4)_{6-y}B_yA_z(OH)_{2-z}$$

wherein M is a cation different from Ca^{2+}, B is an anion different from PO_4^{3-}, A is chosen from the group consisting of O^{2-}, CO_3^{2-}, F^- and Cl^-, x is a number from 0 to 9, y is a number from 0 to 5, z is a number from 0 to 2, wherein the said numbers may also be fractional, with the proviso that the sum of the charges of the Ca and M cations is equal to the sum of the charges of the PO_4^{3-}, B, A, and OH^- anions,
having average size of the crystallites comprised between 0.5 and 200 nm,
for the production of preparations for odontostomatologic applications, useful for the restoration and the protection of dental tissue and, specifically, for the therapy of dentine hypersensitivity and for the remineralisation of enamel and dentine. Specifically, the said nanostructured materials may show lattice deformations and defects.

As it is known, apatites represent the main inorganic process of calcification of normal tissue (i.e. enamel, dentine, cement, bone) and are found associated with other phosphatic and non-phosphatic minerals in pathological calcifications. The main one of these compounds, i.e. hydroxyapatite or hydroxylapatite (HA), having the stoichiometric formula $Ca_{10}(PO_4)_6(OH)_2$ (or $Ca_5(PO_2)_3OH$), and being - in its synthetic (biocompatible) form - the apatite material most widely exploited at a commercial level for several indications in dentistry, orthopaedy and maxillo-facial surgery, is never found in a pure state in the biological tissues. This is due to the possible isomorphous replacements of the Ca^{2+}, PO_4^{3-} and OH^- ions. The calcium ion may be totally or partially

replaced by a number of cations generally (but not exclusively) having oxidation number +2; the phosphate ion (site B) may be replaced by carbonate, acid phosphate, pyrophosphate, sulphate, aluminate and silicate ions, and the hydroxyl ion (site A) may be replaced by halogenide, carbonate and oxide ions.

In comparison with the value 1.67 of the stoichiometric hydroxyapatite, biological apatites have a Ca/P molar ratio comprised between 1.53 and 1.74 (in particular, comprised between 1.53 and 1.64 for the dental enamel, between 1.62 and 1.68 for the dentine and between 1.72 and 1.80 for the bone). In general, the oscillations in the Ca/P ratio may be caused by: 1) lattice vacancies; 2) ions isomorphically replaced or adsorbed on the lattice surface (for instance, the substitution of the PO_4^{3-} anion with acid phosphate, which is divalent, brings about a reduction in the calcium contents); 3) coexistence or presence of possible precursors consisting of phosphates with different Ca/P ratio. Among the latter, in particular, there may be mentioned β-tricalcium phosphate (β-TCP, i.e. $Ca_3(PO_4)_2$, an orthophosphate also known as tribasic calcium phosphate), amorphous calcium phosphate (ACP), octocalcium phosphate (OCP, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$), dicalcium phosphate dihydrate (DCPD, $CaHPO_4 \cdot 2H_2O$, also known as calcium acid phosphate, dibasic calcium phosphate or dicalcium orthophosphate). As it may be deduced from the corresponding formulas, the Ca/P molar ratio values for some of the phosphates considered are as follows:

HA	$Ca_5(PO_4)_3OH$	1.67
β-TCP	$Ca_3(PO_4)_2$	1.50
OCP	$Ca_8H_2(PO_4)_6 \cdot 5H_2O$	1.33
DCPD	$CaHPO_4 \cdot 2H_2O$	1.00

More correctly, the biological apatites may be described as non-stoichiometric carbonatoapatites containing, in general as impurities, different ions. For this reason, biological apatites have variable morphology, crystalline characteristics, chemical and phisico-chemical properties.

Among the possible phosphates and apatite materials, either naturally occurring or obtainable by synthesis, suitable for the production of materials

for use in the odontostomatologic and biomedic fields, hydroxyapatite [$Ca_5(PO_4)_3OH$] has already been presented as the most widespread material. In its structure, the phosphate and calcium ions are placed approximately according to a hexagonal prism; in the direction of elongation (crystallographic axis *c*) the prism is crossed by a channel having a diameter of 3-3.5 Å, housing any OH^- groups or other possible replacing ions (e.g., fluorine and chlorine). HA may crystallise in two forms: a monocline form (spatial group $P2_1/b$) and a hexagonal form (spatial group $P6_3/m$). In the monocline form, a binary symmetry axis is present along the axis *c*, while in the hexagonal form the symmetry axis becomes hexagonal.

Some Authors have hypothesised for the biological samples a simultaneous presence of amorphous calcium phosphate (ACP) and of scarcely crystalline hydroxyapatite. These two phosphates would differ from each other in the intensity of the diffracted X-rays, while having the same broadening of the peaks. The same Authors hypothesised that the bone tissue contains both phases, the first one to be deposited being ACP; with time, the latter phase would undergo a transformation into microcrystalline HA according to a partial solubilisation process, with subsequent renucleation. The transformation process of ACP is apparently controlled by environmental factors (ATP, pyrophosphates, Mg^{2+}, etc.), which stabilise with their presence one of the two phases.

Also octocalcium phosphate [$Ca_8H_2(PO_4)_6 \cdot 5H_2O$] (OCP) has been found in biological samples. Its crystalline structure appears to be very similar to the structure of hydroxyapatite. Actually, OCP may be transformed into HA through a simple chemical mechanism such as the *in situ* hydrolysis of the already formed crystals. This kind of transformation, considered to be irreversible, allows foreign ions to be incorporated in the crystal lattice. Such process might also be controlled by a "layer by layer" transformation, meaning that as soon as OCP is deposited in a layer of the thickness of a unit cell, this is hydrolysed and transformed into two layers consisting of two unit cells of HA. OCP may be considered to be a mediator between the aqueous phase and the apatitic phase. The kind of product obtained (i.e. OCP or HA) depends

on the relative rates of the OCP precipitation process and of the transformation thereof into HA.

As concerns the possible cation replacement in the apatite formula, although this problem has been studied in the literature, the only information of some interest is that the solid solutions are structurally more ordered when the size of the replacing cation is large. It is not possible to theoretically foresee the ability of calcium of being replaced by chemically and crystallographically similar ions; in addition, also when this is the case, the extent of the isomorphous replacement may be partial. However, it is ascertained that the preparation method has a substantial influence on the extent of the replacement.

In an aqueous environment and under conditions similar to the physiological ones, the Mg^{2+} ions inhibit the hydroxyapatite precipitation and promote the formation of β-tricalcium phosphate. Actually, when the magnesium concentration exceeds 10% the simultaneous formation of HA and β-TCP occurs, the latter being the only product that precipitates when the concentration exceeds 25%. The fact that magnesium replaces calcium in β-TCP is made evident by the displacement of the X-ray diffraction peaks in the powder diffraction spectra of the same samples.

However, the magnesium contents in biological apatites is very low (about 1%), and is limited to a surface deposition. This is evidenced by the fact that during the initial dissolution stage of biological apatites (both bone apatite and tooth enamel) a prevailing release of magnesium ions may be observed. In the apatites with magnesium replacement, the lattice parameters show a slight contraction, and the infra-red (I.R.) spectrum bands are shifted towards lower frequencies, in accordance with the lower atomic mass of magnesium and with the different energy of the Mg-O interaction.

Strontium is present in biological apatites only at the impurity level, and may replace calcium, thus causing an expansion of the *a* and *c* axes. The presence of this element in apatites for odontostomatological use is considered to be important in connection with a possible cariostatic effect thereof (in addition to the hypothesised effect of reduction of the dentine sensitivity), and

confers on the apatite a lower solubility and a higher resistance to thermal treatments.

As concerns barium, this cation does not isomorphically replace calcium in the whole concentration interval. As a matter of fact, the variation of the lattice parameters results in an expansion of the cell parameters.

As pointed out before, the basic structure of hydroxyapatite may also be substituted with anionic groups. In particular, in carbonatoapatite the carbonate ion may replace the hydroxyl ion (site A) or the phosphate ion (site B) or both. Although the replacement of the carbonate ion in the site B is preferential in the biological samples, it is also possible to obtain carbonatoapatites of the type A synthetically, by means of high temperature reactions. On the other hand, carbonatoapatites of the type B or mixed type A + B are mainly obtained by precipitation from solutions.

The presence of the carbonate ion in the different sites, which is hardly detectable by X-ray diffractometry, may be evidenced through I.R. spectroscopy, since the position of the absorption bands of the carbonate ion directly depends on the occupied site. The presence of the carbonate ion in hydroxyapatite modifies the lattice dimensions: if the said ion occupies the A site an increase in the *a* parameter is obtained, as a consequence of the greater size of the CO_3^{2-} ion with respect to the OH^- ion; if, on the contrary, the carbonate ion occupies the B site the same parameter undergoes a contraction, due to the smaller O-O distance in the CO_3^{2-} ion with respect to the PO_4^{3-} ion. In addition, it has been observed that the inclusion of the carbonate ion in apatite results in a reduction and in a different morphology of the crystals, which appear to change their shape from a needle-like one to one of comparable length in the various crystal dimensions. The solubility increases as well, while the thermal stability decreases.

In fluoro- and chloroapatites the F^- and Cl^- ions, respectively, replace the hydroxy ion; the said replacement, theoretically, may be total. Fluoroapatite is characterised by an increase in the crystal dimensions, by a decrease in the *a* parameter of the unit cell, by a lower solubility and by an enhanced thermal stability. The lower solubility, and therefore the higher lattice stability,

of the fluoroapatites substantiates the present use of the fluoride ion in the therapy of bone affections and of dental caries.

Chloroapatite is characterised by an expansion of the *a* side and a contraction of the *c* side of the unit cell. The different lattice behaviour of chloroapatite with respect to fluoroapatite derives from the remarkable difference in ionic radius between the two halogens: in fluoroapatites the fluoride ion is placed on the senary axis located on the plane defined by the three calcium ions, while in chloroapatites the chloride ion is slightly displaced from the plane of the metal ions. This phenomenon results in the above-mentioned parameter variations of chloroapatite, while the crystallinity does not seem to be significantly affected, even if the thermal stability decreases. It is possible to observe the presence of solid solutions between fluoroapatites and hydroxyapatites and also between chloroapatites and hydroxyapatites in the whole concentration range; solid solutions may also occur between fluoroapatites and chloroapatites.

The non-stoichiometric character of biological apatites may also be caused by the presence of the acid phosphate ion HPO_4^{2-}; this ion is contained specifically in the tooth enamel, in amounts comprised between 5% and 15%, and is effective in increasing the hydroxyapatite solubility. The presence of acid phosphate in biological and synthetic apatites is not easily detectable, since the carbonate ion, which is almost always present, overlaps the I.R. absorption bands and causes a similar variation of the cell parameters. The original presence of HPO_4^{2-} may be evidenced by the pyrophosphate formation, obtained by heating the apatite samples between 400 e 500°C.

According to some specific embodiments of the invention, therefore, the M cation is chosen among the following ones: H^+, Na^+, Mg^{2+}, K^+, Sr^{2+}, Ba^{2+} and Fe^{2+}, and preferably the x value is comprised between 0 and 2. The B anion is chosen, preferably, among CO_3^{2-}, HPO_4^{2-}, HCO_3^-, and $P_2O_7^{4-}$, while the y value may be comprised, for instance, between 0 and 2. As far as the A anion is concerned, according to a specific choice the latter is absent, the z value being equal to 0 (hydroxyapatites), while according to another

specific choice the A anion is F^-, with z = 2 (fluoroapatites).

The nanostructured apatites according to the invention may be produced by any one of the several known methods, already in use for the production of nanocrystalline materials, such as the synthesis methods from atomic or molecular precursors (e.g., chemical or physical vapour deposition, condensation in gas, chemical precipitation, reactions from aerosol), the methods of production from mass precursors (e.g. by mechanical attrition, by crystallisation from the amorphous state, by phase separation), and the methods borrowed from nature (i.e., biologically mimicked systems).

The conventional deposition of layers of material by electrolytic processes or by vapour condensation has been exploited in recent times to deposit materials of nanometric dimensions with remarkable control and accuracy. The new or improved methods include, in particular, increasingly advanced mono- or multi-bath systems for electrodeposition and new chemical or physical vapour deposition methods, such as molecular beam epitaxy (MBE), metal-organic chemical vapour deposition (MOCVD) and chemical vapour synthesis (CVS). Such methods not only afford an accurate control on the chemistry and the thickness of the layer deposited on a nanometric scale, but they also afford, in some cases, to control the nature of the interfaces within the same layers (L.E. McCandlish, D.E. Polk, R.W. Siegel, and B. H. Kear, Multicomponent Ultrafine Microstructures: Mater. Res. Soc. Symp. Proc. 132 (1989); G. Gumbs, S. Luryi, B. Weiss, and G.W. Wicks. Growth, Processing and Characterisation of Semiconductor Heterostructures: Mater. Res. Soc. Symp. Proc., 326 (1994)).

Several new opportunities also exist in the field of the production of nanophasic materials assembled from atomic clusters synthesised through physical and chemical methods. For instance, chemical precipitation represents one of the conventional methods for synthesising ultrafine powder or colloidal suspensions that has been successfully applied in the synthesis of nanometre-sized clusters with narrow dimensional distribution, for instance by applying the sol-gel technique or the inverse micelle method. Also many high-

temperature gas reaction methods are presently available for the synthesis of nanometric clusters, or of nanostructured powders of bigger size (R.W. Siegel, 1991, *loc. cit.*).

As far as the latter group of techniques is concerned, the synthesis of nanocrystalline materials by *in situ* consolidation, under vacuum, of ultrafine particles condensed in the form of nanometre-sized gas starts when a precursor material, be it an element or a compound, is evaporated into a gas maintained under low pressure, generally well below 1 atmosphere. The evaporated atoms loose energy as a result of the collisions with the atoms or molecules of the gas, and undergo a homogeneous condensation suitable to form clusters of atoms in the highly supersaturated area close to the precursor source. In order to keep the clusters dimensions small while reducing to a minimum the growth with further atoms or molecules or the coalescence among the clusters, it is necessary to rapidly remove the previously nucleated clusters from the highly supersaturated zone. Since the clusters are already suspended in the condensation gases, this may be readily obtained by providing for the conditions to displace the said gas, for instance by natural convection or by forced circulation. The gas-suspended clusters are then conveyed towards a collecting surface (the collection occurring by thermophoresis) and are then placed in a piston-anvil device for consolidation. It is also possible to carry out, for instance, a direct deposition of clusters as thin films or filaments.

Only three basic parameters, working in connection with each other, control the atom clusters formation in the gas condensation process described in the foregoing (McClandish et al., 1989, *loc. cit.*), namely: the rate of atoms supply to the supersaturation area wherein the condensation occurs, the rate of energy removal from hot atoms through the condensation medium, i.e. the gas, and (Siegel, 1991, *loc. cit.*) the removal rate of the previously nucleated clusters from the supersaturation area.

The mechanical attrition method (i.e. lattice destabilisation) allows to produce nanostructures, rather than by cluster coalescence, through the decomposition of coarse-grained structures, caused by a severe mechanical

deformation. The nanometre-sized grains form a nucleus within slip bands of highly deformed precursors materials, thus transforming a coarse-grained structure into a nanophasic one. A great deformation is normally obtained by means of a high energy crushing, but it may also occur as a consequence of surface wear phenomena (E. Hellstern, H.J. Fecht, Z. Fu and W.L. Johnson., J. Appl. Phys., 65 (1989); C.C. Koch, Nanostructured Mater., 2, 109 (1993)), or it may be obtained through other methods of introduction of high deformation densities (R. Valiev, in Mechanical Properties and Deformation Behaviour of Materials Having Ultra-Fine Microstructures, M. Nastasi, D.M. Parkin, and H. Gleiter. 303 (1993)). In the course of the hard mechanical work on the precursors it is also possible to react different materials so as to have them form new phases and compounds.

This quite direct and relatively easy method offers a ready access to the ultrafine grain dimensions useful for the purposes of the invention, and allows to produce commercial amounts of material. The method has been used for the synthesis of the nanostructured apatite materials according to the invention that underwent the comparative experimentation described further on. The nanostructured apatite material according to the invention was produced starting from apatite with microcrystalline structure through lattice destabilisation in a controlled environment, under high energy, by subjecting the starting material to a high mechanical energy transfer treatment. The latter is carried out into a cylindrical reaction chamber by means of high energy impacts from hard balls contained within the same chamber. The kinetic energy of the balls is generated by a rotation movement of the chamber with respect to its main axis and by a revolution movement of the same with respect to an axis parallel to the main one. The transformation of kinetic energy into mechanical energy of lattice destabilisation occurs through impacts between the balls and the starting material. The chamber may work either in air or in inert gas or under vacuum, down to a pressure value of 10^{-6} torr, or with liquids (alcohol, ethers, oils and other organic molecules).

The above method reduces the crystallites dimension through subsequent introduction of lattice defects (which may be evaluated by means of X-

ray diffraction techniques). In particular, the nanoapatite material obtained by the disclosed synthesis method and employed in the applications reported herein shows an average dimension of the crystallites of about 15 nm, with an average strain ranged between 10^{-5} and 10^{-2}.

The apatite-based nanocrystalline products according to the present invention represent a material that simulates the dentine composition and is perfectly compatible, both biologically and structurally, with the dental tissue. The material is able to efficiently become stably integrated with the dentine, thus making it totally impermeable. The dimensional features, the properties of surface activity and the defective contents of the nanoapatites, preferably obtained according to the previously disclosed method, allow to easily obtain both the mechanical filling of the tubules and the fixing reaction with the tubule surface (i.e. remineralisation). Therefore, the dentine sensitivity resulting from the hydraulic conductivity through the tubule structure of dentine is practically removed. As noted in the foregoing, the said permeability is a critical factor in the dentinal pain stimulation theory, as the presence of exposed and open tubules causes, when suitable external stimuli are present, the movement of dentinal fluid, perceived at the neural structure level as a pain stimulus.

According a preferred embodiment of the invention, the nanocrystalline apatite materials are protected from acid attack by subjecting them to a treatment with protective aqueous solutions containing tartaric acid and/or its salts, such as, e.g., aqueous solutions containing potassium sodium tartrate (or Seignette's salt, or Rochelle's salt, $NaOOC(CHOH)_2COOK{\cdot}4H_2O$) and hydrous calcium acetate ($Ca(CH_3COO)_2{\cdot}H_2O$), or, in the alternative, with a solution containing tartaric acid ($HOOC(CHOH)_2\,COOH$). Preferably, a 0.1-1.0 M solution of potassium sodium tartrate and a 0.1-1.0 M solution of hydrous calcium acetate are used in a sequence, by dipping therein the material according to the invention, preferably at 37°C, for periods of time comprised between 5 and 30 minutes. Specifically, the nanoapatite is immersed in the first solution and kept therein for 5 minutes, then it is withdrawn from the first solution and immediately immersed in the second solution for 20-30 minutes.

The material can also be individually treated with one of the following

agents: mono-, bi-, tri-, tetra-, polycarboxylated calcium gluconate in aqueous solution at a concentration of 0.01-5% by weight, or in alcoholic or hydroalcoholic solution at a concentration in the range from 0.01% and 15% by weight; or with an acetic and/or malic and/or hyaluronic solution of chitosan at a concentration in the range from 5% to 20% by weight; or with a solution of human, bovine, swine, rat or turkey tendon collagen, either isotonic or not, at a concentration in the range from 0.5% to 5% by weight. The said treatments result in a protection of the nanostructured material from acid environments. The said material, however, may also be used without any further treatment.

The invention further concerns compositions for odontostomatologic use comprising the apatite-based nanostructured materials described in the foregoing, together with further possible ingredients and excipients of the same kind as those used in preparations for dental hygiene and for the therapy of the oral cavity. The compositions, that are preferably in the form of toothpaste, paste, gel, suspension or solution, preferably contain from 0.5% to 50% by weight of nanocrystalline apatite material, either treated with protective agents or not. Further preferred features of the compositions according to the invention, with particular reference to oral formulations in the gel form, are specified in the further dependent claims.

Some specific embodiments of the invention are described below for merely illustrative purposes, together with the results of the experimental studies carried out on the proposed nanostructured products, including comparative tests with other non-nanocrystalline apatite materials.

EXAMPLE 1

A microcrystalline carbonate-apatite material, non-stoichiometric as regards the hydroxy ion, was subjected to a lattice destabilisation treatment in a controlled environment, under high energy, by using the reaction chamber described in the foregoing. The resulting product (nanoapatite) is characterised by average crystallite sizes of 15-20 nm and by an average microstrain content comprised between 10^{-4} and 10^{-5}.

A portion of the nanocrystalline apatite thus obtained (which will be referred to in the following as nanoapatite 1 or nano 1) has also been sub-

jected to treatment with tartrate in order to increase its resistance to acid attack in the conditions of use in the oral cavity. To that aim, the nanoapatite was treated with a 0.1 M aqueous solution of potassium sodium tartrate (Seignette's salt, Carlo Erba, Milano) at 37°C for 5 minutes, and then with a 0.1 M solution of hydrous calcium acetate (Carlo Erba, Milano) at 37°C for 20 minutes.

EXAMPLE 2

An apatite material of the same nature and composition as the material of Example 1 was subjected to a similar lattice destabilisation treatment, with a different value of the energetic content transferred. The resulting nanoapatite was characterised by average crystallite dimensions of 10-14 nm and by an average microstrain content comprised between $5 \cdot 10^{-4}$ and 10^{-4}.

Also in this case, a portion of the nanocrystalline apatite thus obtained (i.e. nanoapatite 2 or nano 2) was treated with potassium sodium tartrate and hydrous calcium acetate, according to the same procedure of Example 1.

- Dentine permeability test

The effectiveness of the nanocrystalline apatite products according to the invention in the treatment of dentine hypersensitivity was evaluated in terms of reduction of the hydraulic conductance within the dentine tubules, according to a well established protocol (Pashley, 1990, *loc. cit.*), in agreement with the international literature. In order to carry out such test, sound human molars have been used, extracted for orthodontic reasons from young patients, in order to avoid the problem of sclerotic dentine. Each crown was properly separated from the root and cut in order to obtain a crown segment deprived of the occlusal enamel. After removing the pulp tissue, the crown segment was fixed by means of adhesive onto a Plexiglas base, with the flat surface of occlusal dentine facing upwards. The base was crossed through its entire thickness by a tubular stainless steel segment, projecting into the pulp cavity in order to provide the hydraulic connection, below the Plexiglas base, with the hydraulic conductance measuring device. The latter was made of a simple hydrodynamic device consisting of a set of capillary tubes filled with

deionised water and connected through the tubular stainless steel segment to the pulp cavity. The passage of water from the pulp cavity to the occlusal surface through the dentine was evidenced by the displacement of an air bubble in a graduated microcapillary tube located in the hydrodynamic system.

The tests reported below where carried out, by means of the described equipment, by applying on the exposed dentine surface a carboxymethyl cellulose-based gel, to which the single active ingredients under test were added in turn. Each batch of gel was obtained by dissolving 6 g of carboxymethyl cellulose at 50°C in an aqueous solution containing 0.2% by weight of paraben, and stirring the solution until a transparent gel, of the right consistency, was obtained. The various products under test were mixed to 3 ml aliquots of the gel thus produced, according to the following scheme:

1. control, consisting of the only gel vehicle;
2. commercial microcrystalline hydroxyapatite (Merck, Darmstadt, Germany, Art. 2196);
3. commercial microcrystalline hydroxyapatite treated with potassium sodium tartrate and hydrous calcium acetate as described in Example 1;
4. nanoapatite 1, produced according to Example 1;
5. nanoapatite 1 treated with potassium sodium tartrate and hydrous calcium acetate according to Example 1;
6. nanoapatite 2, produced according to Example 2;
7. nanoapatite 2 treated with potassium sodium tartrate and hydrous calcium acetate as described in Example 1.

According to the well established procedure referred to in the foregoing, for each test the following passages were carried out:

- Formation of a smear layer by rubbing with abrasive paper under manual pressure, followed by washing in deionised water.
- Treatment with 0.5 M EDTA (ethylene diammine tetraacetic acid, Sigma, St. Louis, USA) at pH 7.4 for 5 minutes. The permeability was measured after leaving to stand for 2 minutes and subsequently washing the sample. The value so obtained was taken as a reference for the following tests, by

associating it to a permeability of 100%.

- Treatment with the control gel or the gel containing the apatite under test (either treated or non treated) by brushing the tooth for 3 minutes. The said time has been chosen with reference to the correct brushing time in the normal toothbrushing operation. The tooth was painted rather than brushed, in order to avoid forming a smear layer, which would interfere with the evaluation of the product activity.
- Etching with 37 wt. % ortophosphoric acid (Merck, Darmstadt) for 1 minute, and washing. The permeability was evaluated after leaving to stand for 1 minute.

The permeability tests were performed seven times for each of the set times (30, 60, 120 seconds) after treatment with each single gel, and repeating each stage of the above procedure.

The results thus obtained are presented in the following Table in respect of all of the procedural steps. In order to evidence the behaviour of each gel in the presence of acid attack, the data corresponding to the evaluation at 60 minutes after the gel application and after treatment with ortophosphoric acid are also presented in the histogram of the attached Figure.

TABLE 1: EXPERIMENTAL VALUES OF DENTINE PERMEABILITY

	After smear layer formation			Treatm. with EDTA	After gel application			After treatment with H_3PO_4		
Time (sec.) →	30	60	120		30	60	120	30	60	120
Control	67.50	67.82	68.56	100.00	76.21	81.19	75.21	114.10	117.34	109.46
Hydroxyapatite	65.46	66.39	67.55	100.00	61.08	50.33	45.90	83.32	78.71	75.66
Hydroxyapatite, treated	69.16	63.99	64.63	100.00	49.77	45.29	42.56	51.65	50.76	47.20
Nanoapatite 1	62.28	64.47	64.11	100.00	44.40	35.92	32.85	47.67	41.63	38.40
Nanoapatite 1, treated	63.31	67.13	65.19	100.00	42.92	37.09	33.02	37.91	33.93	34.02
Nanoapatite 2	62.58	62.14	61.05	100.00	43.57	35.64	31.08	46.17	40.17	38.22
Nanoapatite 2, treated	64.11	62.96	63.37	100.00	39.89	34.45	30.67	38.18	32.18	30.81

Each value is referred, in %, to the EDTA stage taken as 100% permeability

From the observation of the foregoing table the following conclusions may be drawn, which are even more evident from the enclosed histogram: no positive result was obtained with the control (carboxymethyl cellulose gel), as the dentine was extremely permeable, and particularly susceptible to acid attack, so much so that upon treatment with H_3PO_4 the permeability values are above 100% (see Figure).

The use of commercial microcrystalline hydroxyapatite may bring about a partial occlusion of the dentine tubules, with reduced permeability in comparison with the smear layer formation step. However, after the action of H_3PO_4 no detectable resistance is noted. Some resistance is present, on the other hand, if microcrystalline hydroxyapatite is treated with the Seignette's salt and calcium acetate solutions. The behaviour before and after acid attack, with or without the protective treatment, is evident from the enclosed histogram.

With the use of nanoapatites 1 and 2 the dentine permeability is greatly reduced, to the point that the permeability falls from 100% after treatment with EDTA to about 40% after treatment with the nanoapatites, and further improves up to 120 seconds. In this case, even the acid attack does not result in any appreciable negative effect, this being certainly the most interesting data.

The effectiveness of the treatment with nanocrystalline apatite according to the invention is even more evident when nanoapatites treated with Seignette's salt and calcium acetate are used. These not only produce a remarkable reduction in the dentine permeability in the absence of an acid attack, but, most remarkably, afford an even more considerable reduction after the final acid attack by H_3PO_4, with permeability values not exceeding about 35% of the value after treatment with EDTA.

Although the statistic analysis did not evidence any significant difference between nanoapatites 1 and 2, nanoapatite 2 gave a lower standard deviation in the measurements, and therefore a greater reproducibility in the results.

From th experimental results presented in the foregoing it will be

appreciated that the nanostructured apatite materials according to the invention can be validly proposed as biocompatible materials having a marked and prolonged action in reducing the hydraulic conductance within the dentine tubules, and suitable to become stably and deeply integrated in the dentine tissue. Accordingly, the said materials may advantageously be employed, either as such or included in powder, toothpaste, paste, gel, solutions, mouthwash, suspensions, tablet, capsule, resin or cement compositions, in preparations for the protection of dentine, for the therapy of dentine hypersensitivity, for the remineralisation of enamel, dentine, dental tissues and supporting tissues, for the closure of dentine tubules or the reduction of their functional diameter, for the protection of dental pulp and of prosthetic abutments, as well as in preparations for use as bases and liners for dental restorations with amalgam, as cements for endodontic fillings and for dental prostheses, and as orthodontic cements and sealants for enamel and dentine.

The present invention has been disclosed with particular reference to some specific embodiments thereof, but it should be understood that modifications and changes may be made by the persons skilled in the art without departing from the scope of the invention as defined in the appended claims.

CLAIMS

1. Use of apatite-based nanostructured materials of the general formula:

$$Ca_{10-x}M_x(PO_4)_{6-y}B_yA_z(OH)_{2-z}$$

wherein M is a cation different from Ca^{2+}, B is an anion different from PO_4^{3-}, A is chosen from the group consisting of O^{2-}, CO_3^{2-}, F^- and Cl^-, x is a number from 0 to 9, y is a number from 0 to 5, z is a number from 0 to 2, wherein the said numbers may also be fractional, with the proviso that the sum of the charges of the Ca and M cations is equal to the sum of the charges of the PO_4^{3-}, B, A, and OH^- anions,
having average size of the crystallites comprised between 0.5 and 200 nm,
for the production of preparations for odontostomatologic applications.

2. Use according to claim 1, wherein the said nanostructured materials show lattice deformations and/or defects.

3. Use according to claims 1 or 2, wherein the M cation is chosen from the group consisting of: H^+, Na^+, Mg^{2+}, K^+, Sr^{2+}, Ba^{2+} and Fe^{2+}.

4. Use according to any one of claims 1-3, wherein x is comprised between 0 and 2.

5. Use according to any one of claims 1-4, wherein the B anion is chosen from the group consisting of: CO_3^{2-}, HPO_4^{2-}, HCO_3^-, and $P_2O_7^{4-}$.

6. Use according to any one of claims 1-5, wherein y is comprised between 0 and 2.

7. Use according to claim 1, wherein x, y and z are equal to 0 (hydroxyapatite).

8. Use according to claim 1, wherein x and y are equal to 0, and the A anion is F^-; with z = 2 (fluoroapatites).

9. Use according to any one of claims 1-8, wherein the average size of the said crystallites is comprised between 1 and 50 nm.

10. Use according to any one of claims 1-9, wherein the said preparations for odontostomatologic applications are powder, paste, gel, solutions,

mouthwash, suspensions, tablet, capsule, resin or cement compositions for the protection of the hard dental tissues, the therapy of dentine hypersensitivity, the remineralisation of enamel, dentine, dental tissues and supporting tissues, the closure of dentine tubules or the reduction of their functional diameter and/or for the protection of dental pulp and of prosthetic abutments, or for use as bases and liners for dental restorations with amalgam, as cements for endodontic fillings and for dental prostheses, or as orthodontic cements and sealants for enamel and dentine.

11. Use according to any one of claims 1-10, wherein the said nanostructured apatite materials are treated, in the said production, with protective aqueous solutions containing tartaric acid and/or its salts.

12. Use according to claim 11, wherein a first solution of 0.1-1.0 M potassium sodium tartrate and a second solution of 0.1-1.0 M hydrous calcium acetate are used in a sequence.

13. Use according to claim 12, wherein the said nanostructured apatite material is immersed in the first solution, at 37°C, and kept therein for 5 minutes, then it is withdrawn from the said first solution and immediately immersed in the second solution, at 37°C, and kept therein for 20-30 minutes.

14. Use according to any one of claims 1-10, wherein the said nanostructured apatite materials are treated, in the said production, with protective aqueous solutions containing mono-, bi-, tri-, tetra- or polycarboxylated calcium gluconate at a concentration of 0.01-5% by weight, or in alcoholic or hydroalcoholic solution at a concentration in the range from 0.01% to 15% by weight.

15. Use according to any one of claims 1-10, wherein the said nanostructured apatite materials are treated, in the said production, with a protective acetic and/or malic and/or hyaluronic solution of chitosan at a concentration in the range from 5% to 20% by weight.

16. Use according to any one of claims 1-10, wherein the said nanostructured apatite materials are treated, in the said production, with a protective aqueous solution of human, bovine, swine, rat or turkey tendon collagen, either isotonic or not, at a concentration in the range from 0.5% to 5% by

weight.

17. A composition for odontostomatologic use comprising an apatite-based nanostructured material as defined in claim 1.

18. The composition according to claim 17, wherein the said apatite-based nanostructured material is hydroxyapatite.

19. The composition according to claims 17 or 18, wherein the average size of the crystallites of said nanostructured material is comprised between 1 and 50 nm.

20. A composition according to any one of claims 17-19 in the form of a powder, toothpaste, paste, gel, solution, mouthwash, suspension, tablet, capsule, resin or cement.

21. The composition according to claim 20 in the form of a paste, a gel, or a suspension, containing from 0.5% to 50% by weight of the said apatite-based nanostructured material.

22. A composition according to claim 21 in the form of a gel, wherein the said gel contains a mixture of polyacrylic acid and/or derivatives thereof and polyethylene glycol, or a mixture of polymethacrylic acid and/or derivatives thereof and polyethylene glycol, or a mixture of different polyethylene glycols.

23. The composition according to claim 22, wherein the said gel also contains glycerol.

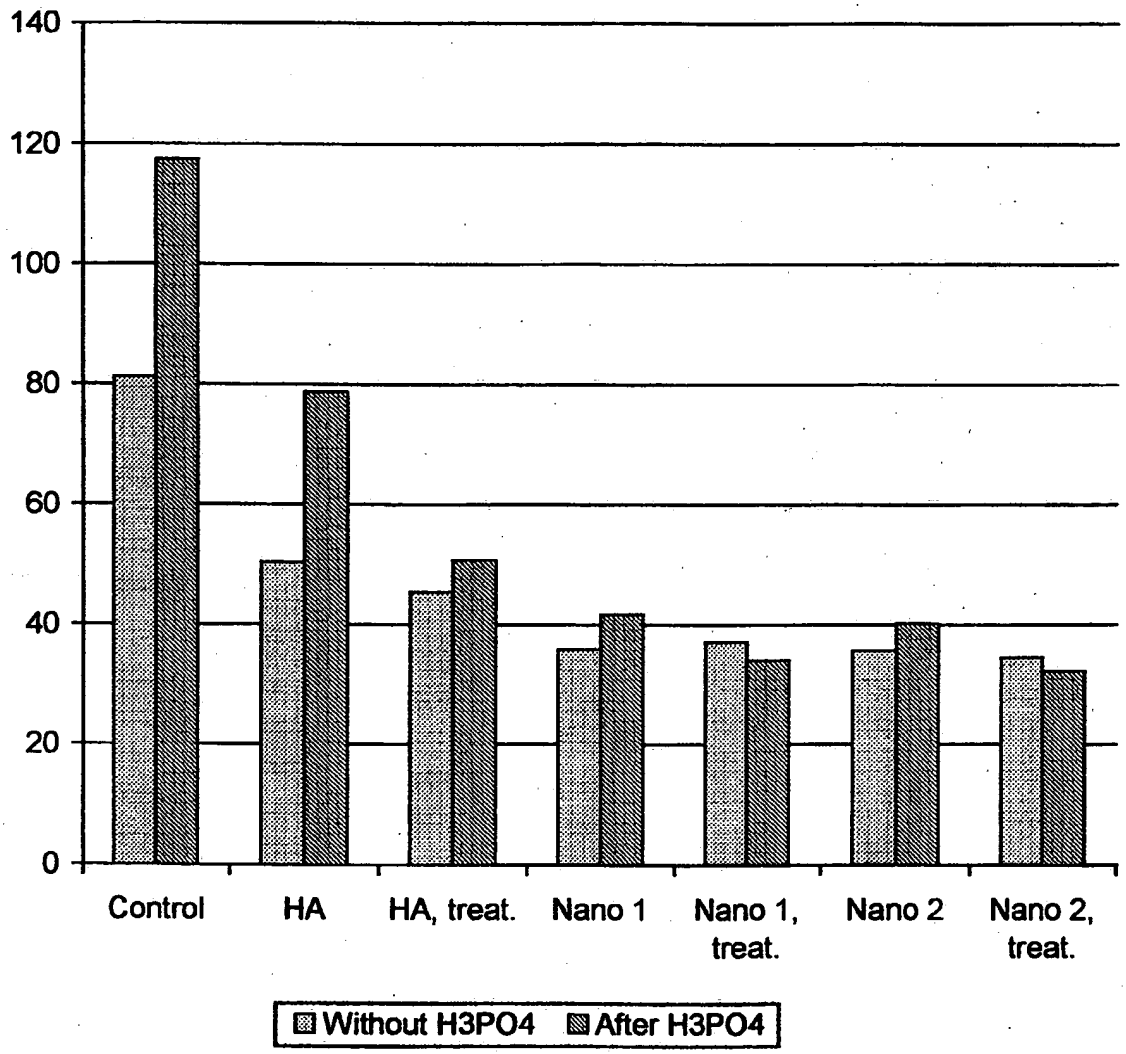
24. A gel composition according to claims 22 or 23, wherein the said apatite-based nanostructured material is contained into polyethylene glycol microspheres.

25. A composition according to claim 21 in the form of a gel, wherein the said gel contains one or more of the following gel-forming ingredients: carboxymethyl cellulose, hydroxymethyl cellulose, optionally substituted chitosan.

26. A composition according to claim 21 in the form of a gel, wherein the said gel is a hydrogel obtained from hydrophylic polymers of the polyvinyl or polysaccharide type, mixed with glycerol and/or polyethylene glycol.

27. A composition according to any one of claims 17-26, wherein the

said nanostructured apatite material is protected by treatment with aqueous solutions containing tartaric acid and/or its salts, or with aqueous, alcoholic or hydroalcoholic solutions containing mono-, bi-, tri-, tetra- or polycarboxylated calcium gluconate, or with acetic and/or malic and/or hyaluronic solutions of chitosan, or with aqueous solutions of human, bovine, swine, rat or turkey tendon collagen.


140
120
100
80
60
40
20
0
Control
HA
HA, treat.
Nano 1
Nano 1, treat.
Nano 2
Nano 2, treat.
Without H3PO4
After H3PO4